High frequency studies of the interaction of micro-nanoparticles with proteins and pathogens

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1. INTRODUCTION

Electromagnetic spectrum from 1 MHz to 100 GHz has significant application in understanding the biological systems because of the ability of these frequencies to get transmitted, absorbed and reflected through biological substances. The Microwave frequency spectrum of 300 MHz to 10 GHz received more attention in the literature due to its better absorption characteristics of the biological material. The interactions of electromagnetic waves with biological material depend upon the tissue boundaries, body size and their dielectric properties [1]. The effect of microwaves can be categorized in two ways: thermal and non thermal effect. The thermal effects of microwaves allow uniform heating, decreased processing time and provide input in the area of food industries and cancer treatment in medical field. The non thermal effect of microwaves has a potential to study the dynamics of biological materials deeply.

The proposed research work aims at understanding behaviour of proteins and pathogens at microwave frequency. It has been found that 37.7 million Indians are affected by waterborne diseases and 1.5 million children die of diarrheal disease alone due to the presence of *E.coli* bacteria in water [2]. Researchers have developed many techniques such as optical, nucleic acid detection, molecular whole cell recognition technique, enzyme substrate method, electrochemical method etc. to quantify the bacteria [2]. In many published studies, it was found that microwaves produce a major effect on the bacteria, which can vary from killing to enhancing the growth of bacteria. For minimizing the cost and detection time, researchers prefer many microwave techniques [3-5]. Recently Wahab, Pugazhendhi and Abdel-Raouf found the antimicrobial effect of nanoparticles while interacting with bacteria [6-8]. These findings show that nanomaterials assisted microwave exposure can lead to selective elimination of pathogens. In order to understand the effect of external alternating electric field on the bacteria, Kunal Samantaray worked on the rod shaped pathogen (Salmonella) suspension using the confocal

microscopy [9] and found that in case of low electric field strength, individual bacteria aligned in the direction of electric field and at intermediate electric field bacteria aligned in such a way that it formed 1D chains in the direction of applied electric field. On increasing the field strength these 1D chains assembled to form 2D array. This effect is related to the quorum sensing of bacteria which affects the cell location and density of bacteria. This can be understood using the diffusion charge around the cell wall of bacteria and polarization effect on bacteria. Quorum sensing of bacteria allows to resolve their social and physical environment. It also involves self-produced extracellular chemical signals which can accumulate in a local environment and are capable of activating transcription of specific genes. One interesting example of related research on quorum sensing is by Whiteley et al. where *Staphylococcus aureus* was shown to use small cyclic peptide pheromones to activate genes for the production of extracellular toxins [10].

The absorption of electromagnetic fields by the microbes depends upon the dielectric properties and chemical composition of micro organism [11]. A.S Anoux, Harris, Christine M. et al. presented permittivity measurement in the microwave range for understanding the lactic acid fermentation in bacteria [12, 13]. Permittivity can be a powerful physical quantity to detect metabolic events in micro organism.

Many interesting studies related to characterization of proteins at microwave region have been published where researchers have calculated the dielectric properties, relaxation time of proteins and their effect on solvent, ligands, pH and temperature [14-24]. To understand the effect of microwaves on the dynamics of denaturation and folding of proteins, Bohr et al. worked on globular proteins such as beta-lactoglobulin by measuring the state of optical polarization of denatured and folded proteins and by perturbing the system with microwaves of frequency 2.45 GHz, he found that microwaves can enhance the kinematics of proteins [25]. Microwave technique can be a promising technique for the synthesis and aggregation of proteins and other biological systems as well. Not only AC electric field, but also DC electric field affects the

protein. In this direction Al-Haq et al. designed apparatus in which they found that DC electric field can induce protein crystallization [26].

The aim of the research work is not only limited to the high frequency characterization of bacteria and proteins but also in understanding the biological systems and their interaction with micro-nanoparticles at high frequencies.

Nanomaterials have facilitated useful and diverse range of applications in the biomedical, industrial, agriculture and food technology field [27-33]. Binding of proteins to the nano particles lead to the understanding of the true nature of the nano particles mediated biological effects and this intern can help in modifying the behaviour and structure of the adsorbed proteins and other molecules [34]. Insulin, a protein and also a drug for the management of diabetes can be stabilized using zinc nano particles [35]. ZnO nano particles can be used as carriers for the delivery of small molecules such as drugs, proteins and genes in the human body due to their small size and large surface to mass ratio [34]. It is evident from the literature that fabrics treated with ZnO nano particles show higher antibacterial activity in comparison to fabric treated with bulk ZnO. So, ZnO nano particles can be used to make an antimicrobial textile [49]. The bio activity of nano sized particles is more in comparison to bulk particles due to large surface area to volume ratio. A large number of nanosized particles can easily cover the bacterial colonies and then by chemical process a large number of active oxygen species are released from ZnO around the bacterial colonies which damage the cell membrane of the bacteria [49]. Extensive literature suggests that ZnO nano particles can be used as an active agent to kill cancerous T-cells. ZnO nano particles generate active oxygen species which induce the apoptosis process (cell death) in the cancerous T-cells.

Microwave techniques have been reported in the characterization of nano particles. Mingzhong et al. characterized the Co/(SiO2) nanoparticles by measuring the microwave permeability in 0.1-18 GHz range where they found that cobalt (Co) particles exhibit high permeability and low magnetic loss [36]. In continuation Nawaf N. Al-Moayed et al. characterized six different

compounds of nano-ferrite powder (FeO, CuFeO, CuFeOZn, FeNiOZn, BaFeO, and SrFeO) by waveguide transmission method and measured the complex permeability and permittivity from 3.95 to 5.85 GHz. Ni and Zn substituted nano spinel ferrites demonstrated a low loss in their permittivity and as well as permeability in comparision to FeO and CuFeO powders. This type of microwave characterization can be a key parameter to compare the different classes of nanoparticles[37]. Microwave characterization technique can assist in understanding the response of nanoparticles and their interaction with biological systems.

2. MOTIVATION

Present research work is the extension of my M.Phil work, where some interesting results were observed due to interaction of ZnO nanoparticles with bacteria and protein. It was observed that the interaction mechanism of nanoparticles was system specific and can lead to far reaching applications in healthcare with deeper understanding of the response of different biological systems to nanoparticles.

3. OBJECTIVES

The proposed research plan can be divided in two major objectives:

1. **Understanding the interaction of micro/nanoparticles with proteins and their effect on the denaturation by measuring electrical properties at microwave frequencies.**
2. **Understanding the pathogen-pathogen electrical signalling and the effect of micro/nanoparticles on the signalling through quorum sensing.**

4. LITERATURE REVIEW

❖ REVIEWS ON BACTERIA

Bacterial pathogens are a major concern in the area of food safety and public health. Their accurate identification and detection is a major issue. Researchers are coming up with different types of detection technologies for accurate measurement of pathogens, they are also trying to

inhibit the microbes using nanoparticles. To analyze the interaction mechanism of bacteria with microwaves, Frank S Barnes and Chia lun J.Hu presented a theoretical model in which they described the mechanism of non thermal effects of RF and microwaves on the biological material [38]. Palaniappan *et al.* discussed various methods of non thermal, electrical treatment of microbes [39]. Fleming in 1944, Goldblith in 1967, Vela and Wu in 1979 carried the experiments and calculated the activation rate of bacteria, fungi and bacteriophages at 2.45 GHz. While performing the experiment Dreyfuss and Chipley in 1980 found the metabolic enzyme activity in *S.aureus* at 2.4 GHz. In the same year Chipley reported that microwave can be a key parameter to enhance or kill the microbes. The absorption of electromagnetic exposure by the microbes depends upon the dielectric properties and chemical composition of micro organism. A. S. Arnoux reviewed the work on permittivity measurement for understanding the lactic acid fermentation in bacteria. Permittivity can be a powerful physical quantity to detect metabolic events [12]. Researchers have developed many techniques such as optical, nucleic acid detection, molecular whole cell recognition technique, enzyme substrate method, electrochemical method etc to quantify the bacteria. In many published studies, it was found that microwaves produce a major effect on bacteria which varies from enhancing the growth of bacteria to killing the bacteria. For minimizing the cost and detection time researchers have preferred many microwave techniques for detection of pathogenic bacteria. These microwave techniques are cost effective, easy to handle, consume less chemicals in comparison to other techniques. In the field of microwaves people have developed many different kinds of microwave sensors in which we can detect the bacteria in less time and at low cost [2-5,40]. Jing, Gaoshan developed a cavity based microwave sensor to detect pathogenic mycobacteria [3]. Nakouti et al. presented a promising microwave substrate based design for rapid detection of bacteria [4]. Sudhi Oberoi also reported dielectric properties of *E.coli* using the microwave cavity [5]. Many studies show that microwaves can enhance the growth of bacteria and can also be the reason of killing, this interaction depends on its energy [41]. Kunal Samantaray reported the effect of external alternating electric field on rod shaped bacterial (*Salmonella*) suspension using confocal microscopy [9] and found that in case of low electric field strength, individual

bacteria aligned in the direction of electric field and at the intermediate electric field bacteria aligned to form 1D chain in the direction of applied electric field. On increasing the field strength these 1D chains assembled to form 2D array. This is related to the quorum sensing of bacteria which affects the cell location and density of bacteria. This can be understood using the diffusion of charge around the cell wall of bacteria and polarization effect on bacteria. Quorum sensing allows bacteria to resolve their social and physical environment. It also involves self-produced extracellular chemical signals which can accumulate in a local environment and are capable of activating transcription of specific genes. Flavien Pillet reported that cell wall can act as a target element to inhibit the growth of bacteria and studied bacterial death due to electro permeabilization by applying pulsed electric field in the range of 6.5 to 8 V/cm [42]. Antimicrobial nature of various metal oxide nano particles was also studied during literature review [43] [44]. Researchers are not only limited to the detection of pathogenic bacteria, but also they are characterizing the bacteria with the aid of nanomaterial and extracting the antibacterial behavior of nano particles [44] [45].

Dunya Zituni reported the effect of low current DC field on the *S.aureus* and *E.coli* and found that inhibition zone of *S.aureus* was higher against *E.coli*. They also reported that *S. aureus* were more sensitive to increasing electric field in comparison to *E.coli*. Electric field treated *S.aureus* gave more structural changes as compared to *E.coli* which is due to the leakage of cytoplasmic material from the bacterial cell [47]. Jaishri Sharma et al. reported electrochemical impedance of pathogenic bacteria in which they detected the bacteria by knowing the variation in the capacitance and resistance of bacteria [48].

❖ REVIEW OF PROTEINS

The principle constituent of most biological cells is free water. Free water contains electric dipoles which change their orientation continuously in microwave radiation. The above fact emphasizes that the biological object exhibits resonating behaviour at microwave frequency. Many studies related to characterization of proteins at microwave region have been published by several researchers. Jeffries Wyman in 1931 was first to report dielectric study of protein solution using resonance method. These measurements were done at 5 MHz according to him

the protein molecules contain a large number of polar groups [14]. G.H. Haggis carried out dielectric measurements of protein solution and measured relaxation time of protein molecule at a frequency of the order of MHz [15]. Strated was the first to find the dielectric properties of insulin. M. Kent reported the dielectric behaviour of hydrated protein powder at 9 GHz [16]. Laogunt et al. had developed a mathematical model to measure the relative permittivity of insulin using the capacitance method over 0.2 to 50 MHz [17]. S. Gabriely et al. had reported the dielectric behaviour of biological tissue in the frequency range 10 to 100 GHz [18].

Jed W Pitera had reported the dielectric properties of proteins and their effect on solvent, legands, pH and temperature using Fröhlich-Kirkwood dipole moment fluctuation model [19]. Joseph Wang et al. fabricated micro thick film for dielectric measurement of insulin [20]. Yifen Wang et Al. had found the dielectric behaviour of protein mixture with change in temperature at the frequencies 27, 40, 915 and 1800 MHz [21]. Abdollah Salimi had reported the electrochemical behaviour of insulin using the glassy carbon electrode [22]. To understand the decay of insulin Ritika Verma et al. had developed a method for the characterization of insulin at microwave frequency. This paper includes the data analysis, activity, permittivity, relaxation time and time delay of insulin at 17 GHz [23].

Researcher are not only limited to the characterizing the proteins, they are also trying to enhance the proper functioning of proteins using nano particles [24].

To understand the effect of microwaves on the dynamics of denaturation and folding of proteins, Bohr et al. worked on globular proteins such as b-lactoglobulin by measuring the state of optical polarization of denatured and folded proteins and by perturbing the system with microwaves of frequency 2.45 GHz, he found that microwave can enhance the kinematics of proteins [25]. Microwave technique can be a promising technique for the synthesis and aggregation of proteins and other biological systems as well. Not only AC electric field, but also DC electric field affects the protein. In this direction Al-Haq et al. designed apparatus in which they found that DC electric field can induce protein crystallization [26].

❖ REVIEW OF NANO PARTICLES

Nanomaterials are a promising technique and are useful in the diverse range of applications such as biomedical field, industrial, agriculture and food technology etc. [49,50-55]. Researchers have synthesized and characterized various nano particles of different shape and reported that these nano particles can act as an active agent in the field of medical diagnostics and therapeutics due to their easy interaction with biological metabolites such as nucleic acids, lipids, proteins and microbes etc. [56]. Recent reviews suggest that researchers gave more preference to metal oxide nano particles in the area of bio engineering, medical diagnostics and therapeutics [50-55]. ZnO is one of the most essential metal oxide nano particles to study due to its vital role in the human body, such as growth and division of cells, ceratogenesis, ontogenesis, immune response and also functioning of pancreases [55]. ZnO nano particles can bind and perturb biological and endocrine systems which are essential for regulating functions of body such as metabolism, digestion, blood pressure and secreting hormones. R. Rajendran et al. reported ZnO nano particles treated fabrics show higher antibacterial activity in comparison to ZnO bulk treated fabrics and antibacterial activity was high against *S.aureus* as compared to *E.coli*. It leads to the idea that Zinc oxide nano particles can be used for producing antimicrobial textiles [49]. Wei Zhao et al. reported that polymer core cell coated over ZnO nano particles when assembled in capsule form of diameter 100 nm act as an active agent to kill the cancerous cells [50]. Shruti R Saptarshi reported the binding of proteins to nano particles to study the nano particles mediated biological effects [34]. Thessicar Antoine et al. reported that zinc oxide tetrapods are effective suppressors and immunoprotective agents against Herpes Simplex Virus 2 (HSV2). They did an experiment on female mice infected with HSV2 and observed that five days ZnO therapy on mice showed lower viral tillers [57]. Imtiyaz et al. studied the cellular and genotoxicity of zinc oxide nano particles in plants and action of these nanoparticles as a bio fertilizer in effective growth of plants and crops [58]. Umrani, Rinku D et al. reported that zinc oxide nanoparticles show antidiabetic activity in streptozotocin-induced type 1 and 2 diabetic rats [59]. The present research work is inspired by the unique tetrapodal shape of zinc oxide

structure which leads to various tremendous applications in the area of medical and biotechnology [33,37].

It is found from the literature that microwave technology not only helps in the characterizing nano particles, but also it plays an important role in determining and utilizing the functions of distinct nano particles in various biomedical and industrial fields [37]. Mingzhong et al. characterized the Co /(SiO2) nanoparticles by measuring the microwave permeability in the 0.1-18 GHz range where they have found that cobalt (Co) particles exhibit high permeability and low magnetic loss [36]. In continuation Nawaf N. Al-Moayed et al. characterized six different compounds of nano-ferrite powder (FeO, CuFeO, CuFeOZn, FeNiOZn, BaFeO and SrFeO) by waveguide transmission method. They measured the complex permeability and permittivity from 3.95 to 5.85 GHz. Ni and Zn substituted nano spinel ferrites demonstrated a low loss in their permittivity and as well as permeability in comparison to FeO and CuFeO powders. These types of microwave characterization can be a key parameter to compare the different classes of nanoparticles [37].

In continuation Encai Hao et al studied the effect of electromagnetic field in the optical region on pairs of silver nano particles which are separated by a few nanometer distance. These pairs exhibit distinct dipoles, quadrupole and higher mutipole plasmonic resonance and these excitations create an external electric field to the particles which is useful in determining single molecule intensities [60].

5. METHODOLOGY:

Proposed doctoral work will be carried out jointly between the Dayalbagh Educational Institute and the Faculty of Engineering at the Kiel University, Germany.

Objectives of the research proposal will be achieved as per the following steps:

I. **Understanding the synthesis and properties of micro-nano structures:**

As part of my M.Phil work it was observed that the tetrapodal micro-structures of ZnO interacted uniquely with proteins as compared to the their spherical part. Therefore, as

first step of the proposed research work the synthesis and the high frequency response of ZnO nanoparticles will be understood.

The nano material functional group at Kiel specializes in the synthesis and characterization of micro-nanomaterials. Recently our co-supervisor Prof. Adelung has introduced a very simple and novel flame transport synthesis (FTS) method. It allows the fabrication of versatile metal oxide nano-micro structures. These metal oxide nano-micro structure synthesized using FTS method have already shown promising applications. FTS method offers a simple way to synthesis metal oxide nano-micro structures with desired porosity. Their mechanical strength is so high that they are even stable at high temperatures (upto1400^{o}c) [61]. In the present research work the preferable method for synthesis is FTS but other methods for the synthesis can also be applied.

- **Work to be carried out at Kiel University:**

  Synthesis of nanoparticles and their morphological characterization

- **Work to be carried out at DEI:**

  High frequency (GHz) electrical characterization and zeta potential analysis of different types of micro- nanoparticles.

II. Interaction of micro-nanoparticles with proteins to understand the denaturation process:

Protein molecules, due to their size and water being the constituent and ambient component, respond to microwave frequencies effectively. The process of denaturation of proteins using microwave technique can be a time efficient way to quantify stability of drugs and in drug designing.

- **Work to be carried out at DEI:**

  Detailed analysis of the denaturation of proteins suspended in different concentrations of **micro-nanoparticles** solution using microwave resonant and transmission technique with low intensity DC electrical perturbations. Zeta

analysis of the solution as a confirmatory test on the stability of protein-nanoparticle suspensions.

- **Work to be carried out at Kiel University:**

  Studying the denaturation of proteins using FTIR method. This method will help in understanding the system from biological perspective and to create parallelism between the results of microwave studies and FTIR method.

III. Measurement of high frequency properties of bacterial cells and the effect of micro-nanoparticles on the signalling pathways of:

Multiplication of bacterial cells happen through conducting signalling pathways, which are sensitive to change in the ambient environment. It is proposed that high frequency study of pathogenic and non-pathogenic species of a bacteria will be carried out. For this cell culturing will be done in the Microbiology Lab of the Department of Botany, Dayalbagh Educational Institute.

- **Work to be carried out at DEI:**

  Cell culturing and study of high frequency electrical properties of bacteria in the presence of micro-nanoparticles of different types (spherical and tetrapodal) in the presence of small magnitude DC and very low frequency AC electric fields. This will help in carrying out quorum sensing by analysing the high frequency response of pathogens.

- **Work to be carried out at Kiel University:**

  The above mentioned electrical characterization will be further studies using gene expression analysis. This study will facilitate in understanding genetic cause behind the electrical response.

6. EXPECTED OUTCOME

Research proposal will lead to following outcomes:

i. Electrical characterization method for studying the denaturation of proteins will lead to a quick method to understand the effect of nanoparticles on the decay of

proteins. In this study particular emphasis will be on insulin protein and stabilization of its decay using micro-nanoparticles. The outcome of this study can lead to effective drug design.

ii. The high frequency method to study the quorum sensing will be a novel method to understand the molecular genetic effects in biological cells. The gene expression and the virulence studies of bacteria-nanoparticle co-culture can have potential application in packaged food due to biocompatibility of micro-nanoparticles. The gene expression analysis of bacteria when cultured with micro-nanoparticles can also give insight into the fundamental mechanism of quorum sensing in bacterial cells.

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